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EFFECT OF DIFFERENT STARTER CULTURE COCOA FERMENTATION APPROACHES ON THE PHYSIOCHEMICAL CHARACTERISTICS

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ABSTRACT

Cocoa fermentations were performed in this study under the following experimental regimens i.e., beans naturally fermented with natural microflora, aseptically prepared beans with no inoculums and beans inoculated with defined cocktail inoculums containing microorganisms at a suitable concentration at appropriate time intervals. Cocoa beans fermentation is an absolute requirement for the full development of chocolate flavor precursors. The culture combinations inoculated into cocoa fermentation processes are - CC1 (S. cerevisiae and L. fermentum), CC2 (L. fermentum and A. aceti), CC3 (A. aceti and S. cerevisiae) and CC4 (S. cerevisiae, L. fermentum and A. aceti) with varying time interval of 0, 24 and 48hr respectively. The result obtained from the physicochemical analysis showed that the acidity value increased with decreasing pH and higher significance difference ($p<0.01$) was observed. The proximate analysis results showed that, the moisture content of the naturally and defined culture fermented cocoa fruit does not show any significant difference ($P\leq0.05$). The protein content was found to be decreasing during the fermentation process. There exists a significant difference ($P <0.05$) in the protein value between the unfermented and fermented samples. The ash content of the cocoa fruit seeds did not show significant difference ($P\leq0.05$) between the unfermented and fermented cocoa fruit seeds. Carbohydrate content tends to decrease during the process of fermentation since the microbes were able to metabolize the carbon source. The result obtained showed significant difference ($P <0.05$) in the carbohydrate value between the unfermented, naturally fermented and define culture fermented sample.

KEYWORDS: Cocoa, Microflora, Carbohydrate & Fermentation

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Original Article

INTRODUCTION

Raw cocoa is harvested from the tree of *Theobroma cacao*, it has to be processed or cured before chocolate production and market. Curing involves different steps like fermentation, drying and roasting (Lehrian and Patterson 1983). The white mucilaginous pulp which covers the beans contains about 14% sugar and 1.5% pectin at pH 3.5. In different geographical locations, the beans are usually stored in large wooden boxes and a vigorous natural fermentation starts. The fermentation lasts for up to 7 days, during which time the beans are turned daily to increase aeration (Schwan *et al*., 1986). During the fermentation, microbial activity in the mucilaginous pulp produces alcohols and acids and liberates heat, and complex biochemical reactions occur within the bean cotyledons due to the diffusion of metabolites from the microorganisms.

Fermentation of the cocoa fruit seed is essential for removing the pulp that envelops the seeds and developing precursors of chocolate flavor. Sugars and polysaccharides of the seed pulp are fermented by

microorganisms, producing metabolites and conditions that cause seed death and initiate an array of biochemical reactions within the seed that generate chocolate flavour precursors. These flavours are fully developed on subsequent seed roasting and conching as part of the chocolate making process. Cocoa fruit seed fermentation is still an uncontrolled traditional process conducted by a consortium of unknown indigenous species of yeasts, lactic acid bacteria and acetic acid bacteria. To transform the fermentation into an efficient industrialized process, controlled by the use of defined starter cultures, it is essential to know how each microbial group contributes to the fermentation process and chocolate quality.

Physical properties of chocolates were influenced by the polymorphism of cocoa butter, which is the major component of solid fat in the chocolate. The control of the molecular structure and polymorphic forms of cocoa butter was particularly important in the manufacture of chocolate (Schlichter *et al.,* 1988). Cocoa butter was responsible for the smooth texture, contraction, flavour release and gloss of chocolate. The acidity of raw cocoa seeds is variations in the conditions during fermentation such as duration affect the acidity of cocoa seeds (Biehl *et al*., 1990). During fermentation acids of the fermented cocoa seeds such as acetic acid can be diffused into the cocoa seed leads to decrease in the pH (Thompson *et al*., 2001). Acetic acid in cocoa seed ranged from 0.38 to 0.92% and affects noticeable acid taste in final product. Ghanaian cocoa seed that renowned as high quality cocoa seed has lower in acid taste (Jinap *et al.,* 1995). First phase of fermentation yeasts possess an intense metabolism favored by the acidity of the fermenting environment. The richness of fermentable carbohydrates and the low oxygen content of the mass favors yeast fermentation metabolism very quickly and consumption of all the simple sugars converted into ethanol and carbon dioxide (Lehrian *et al.,* 1984).

The direct addition of selected starter cultures to raw materials has been a breakthrough in the processing of fermented foods, resulting in a high degree of control over the fermentation process and standardization of the end product (Oberman *et al.,* 1998). Use of functional starter cultures in the food fermentation industry was being explored in a functional starter cultures that possess inherent functional property. Which contributes food safety and organoleptic, technological, nutritional, or health advantages. The implementation of carefully selected strains as starter cultures or co-cultures in fermentation processes could help to achieve in situ expression of the desired property, maintaining a perfectly natural and healthy product (De Vuyst, 2000). A mixed starter culture encompassing strains of *Lactobacillus fermentum*, *Acetobacter pasteurianus* and *Saccharomyces cerevisiae* produced cocoa of a superior quality compared to fermentations inoculated with a pure LAB/AAB bacterial starter culture (Lefeber *et al.,* 2012). Fermentation is an important step in the post-harvest processing of cocoa seeds. Introduction of a specific microbial starter culture improve the quality of the fermentation, mixed culture fermentation produces metabolic products that serve as the precursors for the flavor development process.

MATERIALS AND METHODS

Preparation of Cocoa Seed Fermentation

Natural fermentation was carried out with 10kg of freshly harvested cocoa fruit opened and placed in plastic basket covered with banana leaves and placed for 6 days natural fermentation. Fermentation pH and acidity were measured every day and box was changed every two days interval. After completion of six days fermentation cocoa seeds were sun dried and roasted. The flavor components of fermented cocoa fruits were analyzed.

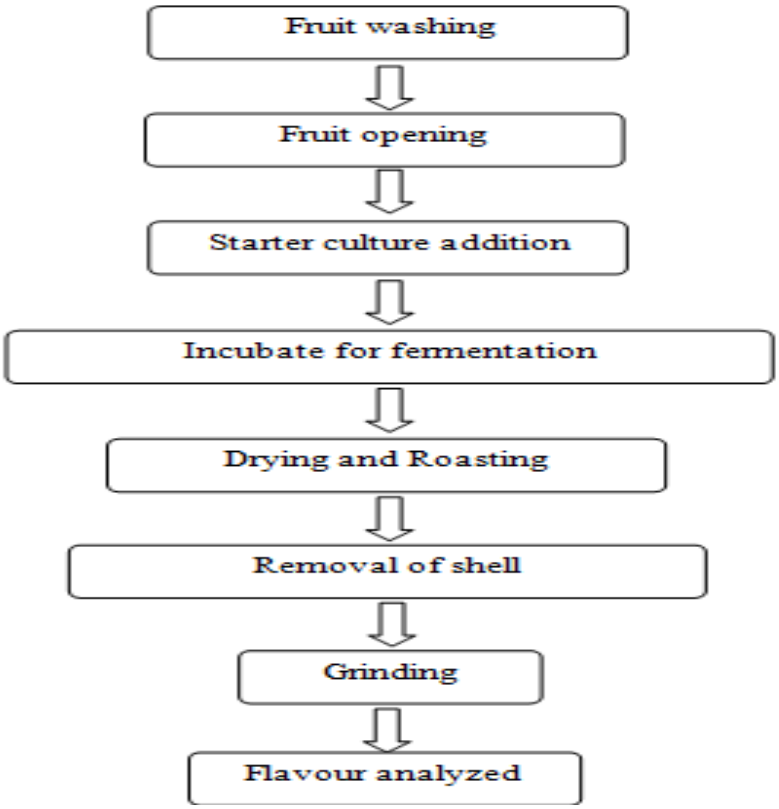

Figure 1: Defined Starter Culture Initiated Cocoa Fruit Seed Fermentation

Determination of Ph and Acidity

5g of cocoa seed sample were drawn from the fermented medium to measure the pH and acidity. Sample was homogenized in 100 mL hot distilled water, stirred manually for 30 s and filtered using Whatman No. 4 filter paper. The pH was measured from filtrated liquid using a pH meter fitted with a glass electrode and an aliquot (25 mL) was used to determinate pH by titration with 0.1 N NaOH solutions (Nazaruddin *et al.*, 2006).

Proximate Analysis

Fermented final cocoa powder were analyzed for moisture (oven drying method), crude fat (Soxhlet extraction method), crude protein (Kjeldahl method) and ash were determined following the procedures in AOAC (2005) methods 931.04, 963.15, 970.22 and 972.15 respectively. Carbohydrate (difference method) was determined using 'by difference' method. All the analyses were performed in six replicate and the mean values reported.

RESULTS AND DISCUSSIONS

Assessment of pH of Cocoa Fruit Seed during Fermentation Initiated with Different Culture Combinations (CC)

The pH of different cocoa fruit seed fermentation viz. Natural fermentation, CC1, CC2, CC3 & CC4 during fermentation period of 6 days at a sampling interval of one day and the results are furnished in Table 1. It was observed that the pH of the fresh cocoa fruit seed gradually decreased during 6 day of fermentation. The pH of fresh cocoa fruit was 6.33 ± 0.010 and it has been reduced to 4.32 ± 0.008 on 6^{th} day of fermentation. The result obtained showed that there is a significant difference in the pH value between the naturally fermented and combined culture fermented cocoa fruit seed.

Table 1: Assessment of pH of Cocoa Fruit during Fermentation Process Initiated with Different Culture Combinations (CC)

Treatments	Duration of Fermentation (in Days)						
	0	1	2	3	4	5	6
NF	6.33 ± 0.010	5.80 ± 0.007	5.33 ± 0.014^{b}	5.13 ± 0.007^{b}	4.78 ± 0.009^{b}	4.53 ± 0.017^{b}	4.32 ± 0.008^{c}
CC1	6.33 ± 0.006	5.79 ± 0.007^{bc}	5.33 ± 0.016^{b}	5.13 ± 0.009^{b}	4.79 ± 0.011^{b}	4.54 ± 0.020^{b}	4.32 ± 0.011^{c}
CC2	6.33 ± 0.010	5.78 ± 0.007^{b}	5.22 ± 0.007^{a}	5.11 ± 0.004^{b}	4.77 ± 0.006^{b}	4.52 ± 0.007^{b}	4.25 ± 0.008^{b}
CC3	6.33 ± 0.012	5.88 ± 0.007^{d}	5.54 ± 0.007^{c}	5.34 ± 0.007^{c}	4.97 ± 0.003^{c}	4.81 ± 0.007^{c}	4.80 ± 0.005^{d}
CC4	6.33 ± 0.006	5.75 ± 0.004^{a}	5.20 ± 0.007^{a}	5.08 ± 0.007^{a}	4.65 ± 0.009^{a}	4.41 ± 0.005^{a}	4.12 ± 0.005^{a}

NF-Natural Fermentation ; CC1- *S. cerevisiae* and *L. fermentum;* CC2- *L. fermentum* and *A. aceti*; CC3- *A. aceti* and *S. cerevisiae;* CC4- *S. cerevisiae, L. fermentum* and *A. aceti*

The physicochemical parameters of pH and acidity of cocoa fruit seed were observed in natural and defined culture combinations from '0' to '6' days. The changes in pH and acidity upto 6 days of fermentation improved the flavour development in final fermented cocoa seeds. The result revealed that the pH value was found to be decreasing during the course of the fermentation for the period of 6 days. The pH developed in cocoa fruit fermentation process with the application of naturally occurring and combined cultures showed that there was no significant difference between the samples. However, as days increased the pH value started to decrease for all the treatments with the lowest pH value of 4.12±0.005 that was observed in the combination of CC4. After 6th day of fermentation, it was found that there was a drastic difference in the pH value between the naturally fermented sample and the defined culture combinations inoculated cocoa fruit samples. Nazaruddin *et al.,* (2006) showed that the pH value decreased from 6.54 to 4.35 during cocoa seed fermentation process which was in agreement with the present study. Decrease in pH was due to diffusion of acids into the seeds, produced by lactic and acetic bacteria during the fermentation process (Thompson *et al.,* 2001).

Assessment of Acidity of Cocoa Fruit Seed during Fermentation Process Initiated with Different Culture Combinations (CC)

The mean ± SE acidity of different cocoa fruit seed fermentation viz. Natural fermentation, CC1, CC2, CC3 & CC4 during fermentation period of 6 days at an interval of one day and the results are furnished in Table 2. It was observed that the acidity of the fresh cocoa fruit seed gradually increased during 6 day of fermentation due to the production of ethanol, lactic acid, acetic acid. The acidity of fresh cocoa fruit is 0.75 ± 0.008 is increased into 2.59 ± 0.015 on 6th day of fermentation. On statistical analysis there was highly significant difference between natural and culture combined cocoa fruit seed fermentation with regard to the acidity. There was a negative correlation between pH and acidity of the cocoa fruit fermentation.

Table 2: Assessment of Acidity of Cocoa Fruit during Fermentation Process Initiated with Different Culture Combinations (CC)

Treatments	Duration of Fermentation (In Days)						
	0	1	2	3	4	5	6
NF	0.75 ± 0.008	1.08 ± 0.004bc	1.16 ± 0.011b	1.18 ± 0.006a	2.32 ± 0.023c	2.45 ± 0.045b	2.59 ± 0.015b
CC1	0.75 ± 0.008	1.05 ± 0.005a	1.16 ± 0.057b	1.19 ± 0.005a	2.25 ± 0.008b	2.44 ± 0.008b	2.62 ± 0.008c
CC2	0.75 ± 0.008	1.09 ± 0.005c	1.17 ± 0.042b	1.20 ± 0.005a	2.35 ± 0.005c	2.50 ± 0.004bc	2.66 ± 0.007d
CC3	0.75 ± 0.006	1.07 ± 0.005b	1.10 ± 0.006a	1.28 ± 0.004b	2.21 ± 0.005a	2.23 ± 0.012a	2.23 ± 0.008a
CC4	0.75 ± 0.005	1.10 ± 0.005c	1.26 ± 0.007c	1.36 ± 0.009c	2.41 ± 0.004d	2.56 ± 0.007c	2.80 ± 0.005e

NF-Natural Fermentation; CC1- *S. cerevisiae* and *L. fermenutm;* CC2- *L. fermentum* and *A. aceti*; CC3- *A. aceti* and *S. cerevisiae;* CC4- *S. cerevisiae, L. fermentum* and *A. aceti*

The result revealed that the fermented cocoa fruit seed acidity was varied between naturally and defined culture combinations introduced into fermentation process. During the first day of fermentation, there was no significant difference between the groups. Acidity value increased from first day to final day of fermentation. In this research, the acidity percentage of different type cocoa fruit seeds had a high ($p < 0.01$) significant increase from 0.75 ± 0.005 to 2.80 ± 0.005 which was the combination of three cultures during six days of fermentation. These results were in conformity with the findings of Jinap and Dimick (1990). Overall results showed that change in acidity is a better indicator of decreasing pH trend for all fermentation treatments. Duncan *et al*., (1989) stated that titra table acidity is a better measure of the total acids in cocoa liquor than pH, and both parameters have been correlated with taste scores for flavour acidity.

Effect of Cocoa Fruit Fermentation Initiated with Different Culture Combination on Proximate Principles

The proximate composition such as moisture, protein, fat, ash and carbohydrate of the different cocoa fruit seed fermentation viz. unfermented (UF) and naturally fermented (NF); CC1, CC2, CC3, and CC4 are given in Table 3. It was observed that the moisture content of unfermented (UF) and naturally fermented (NF); CC1, CC2, CC3 and CC4 were 3.5 ± 0.036, 4.1 ± 0.049, 4.0 ± 0.057, 4.0 ± 0.065, 4.0 ± 0.057 and 4.1 ± 0.061 respectively. The moisture content of the unfermented cocoa fruit seed was found to be lower than the naturally fermented and controlled fermented samples with culture combinations. There was no significant difference ($P \leq 0.05$) existed between the naturally and controlled fermented samples. The difference in the moisture content of the fermented sample when compared with the unfermented sample might be due to the absorption of atmospheric moisture during drying conditions. (Afoakwa *et al.*, 2011) reported the moisture content of the fermented sample to be 4.2 which was in accordance with the present study.

The protein content of unfermented (UF) and naturally fermented (NF) and controlled fermented samples such as CC1, CC2, CC3 and CC4 were 17.6 ± 0.073, 16.9 ± 0.06515, 16.7 ± 0.057, 16.5 ± 0.081, 16.3 ± 0.047 and 5 ± 0.094 respectively. The protein content was found to be decreasing during the fermentation process. There exists a significant difference ($P < 0.05$) in the protein value between the unfermented and fermented samples. The earlier studies reported the similar range of protein value in the fermented samples of cocoa fruit seed by Fapohunda and Afolayan (2012).

The fat content of unfermented (UF) and naturally fermented (NF) and culture combined fermented samples such as CC1, CC2, CC3 and CC4 were 51.2 ± 0.470, 53.6 ± 0.057, 53.3 ± 0.240, 53.2 ± 0.057, 53.4 ± 0.057 and 53.5 ± 0.057 respectively. The fat content of the fermented cocoa fruit seeds was found to be increased when compared to the unfermented seed which was attributed to the removal of water and concentration of fat in the seeds during the process of drying.

The ash content of unfermented (UF) and naturally fermented (NF) and culture combined fermented samples such as CC1, CC2, CC3 & CC4 were 3.3 ± 0.004, 3.3 ± 0.002, 3.3 ± 0.002, 3.3 ± 0.004, 3.3 ± 0.006, 3.3 ± 0.002 respectively. The carbohydrate content of unfermented (UF) and naturally fermented (NF) and culture combined fermented samples such as CC1, CC2, CC3 & CC4 were 24.4 ± 0.081, 22.1 ± 0.047, 22.7 ± 0.093, 23.0 ± 0.093, 23.0 ± 0.061, 23.6 ± 0.054 respectively. Statistical analysis revealed that there was highly significant difference between the control and treatments (UF, NF, CC1, CC2, CC3 and CC 4). The ash content of the cocoa fruit seeds did not show significant difference ($P \leq 0.05$) between the unfermented and fermented cocoa fruit seeds. Carbohydrate content tends to decrease during the process of fermentation which might be due to the utilization of carbohydrate source during the process of fermentation. The result obtained showed significant difference ($P < 0.05$) in the carbohydrate value between the unfermented, naturally fermented and controlled fermented sample. Similar results were observed in the research investigation carried out in cocoa plantation

regions as reported by Afoakwa *et al.*, 2011.

Table 3: Effect of Cocoa Fruit Fermentation Initiated with Different Culture Combination on Proximate Principles

Treatments	Moisture %	Protein %	Fat %	Ash %	Carbohydrate %
UF	3.5±0.036^{c}	17.6±0.073^{e}	51.2±0.470^{b}	3.3±0.004	24.4±0.081^{e}
NF	4.1±0.049^{b}	16.9 ± 0.065^{d}	53.6±0.057^{a}	3.3±0.002	22.1±0.047^{a}
CC1	4.0±0.057^{ab}	16.7±0.057^{c}	53.3±0.240^{a}	3.3±0.002	22.7±0.093^{c}
CC2	4.0 ±0.065^{a}	16.5±0.081^{bc}	53.2±0.057^{a}	3.3±0.004	23.0 ±0.093^{d}
CC3	4.0±0.057^{ab}	16.3±0.047^{b}	53.4±0.057^{a}	3.3±0.006	23.0±0.061^{d}
CC4	4.1±0.061^{b}	15.5 ± 0.094^{a}	53.5±0.057^{a}	3.3±0.002	23.6±0.054^{b}

UF- Unfermented ; NF-Natural Fermentation; CC1- *S. cerevisiae* and *L. fermentum;* CC2- *L. fermentum*and *A. aceti*; CC3- *A. aceti* and *S. cerevisiae;* CC4- *S. cerevisiae, L. fermentum* and *A. aceti*

CONCLUSIONS

The quality of the cocoa fruit seed fermentation with different culture combination was investigated by conducting acidity, pH and proximate composition analysis at a time interval of 24 hr till the end of fermentation. The present study revealed that the developed acidity increased with a decrease in pH during progress of fermentation which was inversely proportional to each other. The statistical analysis of developed acidity in fermented cocoa fruit mass revealed that the acidity and pH showed the higher significance difference ($p < 0.01$). This technique is suitable and can be promoted among local cocoa planters to initiate a starter culture combined fermentation process for better cocoa beans quality. Further study will be focus on the different fermentation approaches can change the effect of cocoa beans flavor and to standardize the fermentation process.

REFERENCES

1. *Afoakwa, E.O. Quao, J. Takrama, J. Budu, A. S. & Saalia, F. K. (2011). Chemical composition and physical quality characteristics of Ghanaian cocoa beans as affected by pulp pre-conditioning and fermentation. Journal of Food Science and Technology,* ***50(6):*** *1097-1105.*
2. *AOAC. (2005). Official methods of analysis, 18th edn. Association of Official Analytical Chemists, Washington, D.C, USA.*
3. *Biehl, B., Meyer, B. Said, M. & Samarakoddy, R. J. (1990). Bean spreading: a method for pulp preconditioning to impair strong nib acidification during cocoa fermentation in Malaysia. Journal of Agriculture and Food Chemistry,* ***51****: 35–45.*
4. *De Vuyst, L., (2000). Technology aspects related to the application of functional starter cultures. Food Technology and Biotechnology,* ***38****: 105–112.*
5. *Duncan, R. J. E., Godfrey, G. Yap, T. N. Pettipher, G. L. & Tharumarajah, T. (1989). Improvement of Malaysian cocoa bean flavour by modification of harvesting, fermentation and drying methods- The Sime-Cadbury Process. Cocoa Grower's Bull,* ***42****: 42-57.*
6. *Fapohunda, S. O., & Afolayan, A. (2012). Fermentation of Cocoa Beans and Antimicrobial Potentials of the pod Husk Phytochemicals. Journal of Physiology and Pharmacology Advances,* ***2(3):*** *158-164.*
7. *Jinap, S., & Dimick, P. S. (1990). Acidc characteristics of fermented and dried cocoa beans from different countries of origin. Journal of Food Science,* ***55****: 547−551.*
8. *Jinap, S., Dimick, P. S. & Hollender, R. (1995). Flavour evaluation of chocolate formulated from cocoa beans from different countries. Food Control* ***6****: 105-110.*

9. *Lefeber, T., Papalexandratou, Z. Gobert, W. Camu, N. & De Vuyst, L. (2012). On-farm implementation of a starter culture for improved cocoa bean fermentation and its influence on the flavour of chocolates produced thereof. Journal of Food Microbiology,* ***30****:379-392.*
10. *Lehrian, D. W., & Patterson, G. R. 1983. Cocoa fermentation. In G. Reed (ed.), Biotechnology, a comprehensive treatise,* ***5****: 529–575.*
11. *Nazaruddin, R., Seng, L. K. Hassan, O. & Said, M.(2006). Effect of pulp preconditioning on the content of polyphenols in cocoa beans (Theobroma Cacao) during fermentation. Industrial Crop and Products,* ***24****: 87-94.*
12. *Oberman, H., & Libudzisz, Z. (1998). Fermented milks. In B. J. B. Wood (Ed.), Microbiology of fermented foods, 1: 308–350.*
13. *Schlichter, J., & Garti, N. (1988). Solidification and polymorphism in cocoa butter and the blooming problems. In: Crystallisation and polymorphism of fats and fatty acids, Ed. by Garti N. and Sato K., Marcel Dekker, Inc., 363-391.*
14. *Schwan, R. F., Vanetti, M. C. D. Silva, D. O. Lopez, A. & de Moraes, C. A. (1986). Characterization and distribution of aerobic, spore-forming bacteria from cacao fermentations in Bahia. J. Food Sci.* ***51****:1583–1584.*
15. *Thompson, S.S., Miller, K. B. & Lopez, A. S. (2001). Cocoa and coffee. Food Microbiology, 721 – 736.*